Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

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Original Paper

Detection of *E. coli* O_{157} and Salmonella species in some raw chicken meat cuts in Ismailia province, Egypt

Fahim A. Shaltout[1], Islam, Z. Mohammed[2], El-Sayed A. Afify[3*]

[1] *Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University.*

[2] *Animal Health Research Institute, ARC.*

[3*] *Veterinary supervisor*

ARTICLE INFO

Keywords

Chicken meat

E. coli O_{157}:H_7

fimA gene

fliC gene

Salmonellae

Received 08/08/2020
Accepted 07/09/2020
Available On-Line 01/10/2020

ABSTRACT

One hundred random fresh raw chicken breast and thigh samples (50 of each) were collected from an automatic poultry dressing plant in Ismailia city, Egypt for bacteriological and genetic detection of *E. coli* O_{157}:H_7 and Salmonella species. Out of the examined 100 samples, only 4 (4%) samples were contaminated with *E. coli* O_{157}:H_7, while non-O_{157} *E. coli* isolates represented 11% of *E. coli* isolates which were serotyped as O_{114}:H_{21}, O_{127}:H_6, O_{126} and O_{26} with incidence of 2, 4, 3, and 2% of the examined samples, respectively. On the other side, Salmonellae were detected in 11 samples (11%) and serologically identified as *S.* Typhi, *S.* Typhimurium and *S.* Enteritidis with the incidence of 1, 3, and 7%, respectively. Further, PCR investigation indicated that *fliC* gene was detected in all 4 isolates of *E. coli* O_{157} with incidence of 100%; while, *fimA* gene in 5 Salmonella isolates was detected in 4 (80%) of such examined isolates. Accordingly, it is obvious that raw chicken meat cuts were loaded with pathogenic foodborne bacteria exposing consumers to the high risk of food poisoning. Moreover, the results cleared that bacteriological traditional methods for detection of bacteria contaminating foods are labor-intensive and time-consuming, but PCR are more rapid and highly sensitive for identification of foodborne pathogens.

1. INTRODUCTION

In recent years, the poultry trade has skilled an incomparable growth rate, which may be attributed to that poultry production has comparatively low prices, highly nutritious, rapid development rates, and an excellent sorts of further-processed products (Barbut, 2015).

Chicken meat may be a common source of microbial foodborne pathogens like Salmonella spp. and *E. coli* (Yulistiani *et al.*, 2019) which can gain access to chicken meat through the whole cycle of production starting with scalding, defeathering and evisceration besides cross contamination which may come from adjacent birds and contaminated equipment. Throughout chicken slaughtering and preparation steps, fecal matter may contaminate carcasses due to evisceration faults (Mbata, 2005). Salmonellae and *E. coli*, as members of Enterobacteriacae, are considered the 2nd most common serious causes of foodborne illness worldwide (FSIS, 2008).

Escherichia coli are a large group of pathogenic and non-pathogenic enteric bacteria. Some pathogenic strains of *E. coli* can cause GIT disturbances through secreting heat-stable Shiga toxin, which commonly detected in the bowel of many animals reared for food production. The most commonly reported type of Shiga toxin producing *E. coli* (STEC) in USA is O_{157} strain. Some types of STEC frequently associated with severe disease, including symptoms of watery bloody diarrhea and some sort of kidney failure known as hemolytic uremic syndrome (HUS) (CDC, 2016).

Salmonellae are widely distributed in nature; they were contributed among the causes of worldwide foodborne pathogens. According to an estimation made in 2010, Salmonellae were involved in more than 80 million cases of foodborne gastroenteritis every year worldwide, of which 155,000 were fatal (Majowicz *et al.*, 2010).

Polymerase Chain Reaction (PCR) is a laboratory investigative and confirmatory method through in vitro amplification of deoxyribonucleic acid (DNA); a sequences that are preferably unique to the organism of interest. Beginning with DNA of any origin (bacteria, viral, plant, animal) PCR can increase the amount of the targeted DNA sequences 10^6 to 10^9 times. Genetic-based diagnostic methods characterized by specificity, sensitivity, and confirmatory assays compared to traditional methods (McKillip *et al.*, 2004). Genes encoding H_7-specific flagellar antigen such as *fli*C gene of *E. coli* is a confirmatory indicator for *E. coli* O_{157}:H_7 (Carey *et al.* 2009). In addition, *Salmonella enterica* food poisoning serovars are characterized by fimbrial genes encoded by *fim* genes, where *fimA gene is* the major subunit (Zeiner *et al.*, 2012). These genes make the virulence and support the bacterial pathogenicity leading to more serious hazards.

* Corresponding author: El-Sayed A. Afify, ssmm1806@gmail.com

Therefore, the present study was conducted to investigate the incidence of *E. coli* O_{157}:H_7 and Salmonellae in the examined chicken meat samples by both traditional and molecular assays.

2. MATERIAL AND METHODS

2.1. Bacteriological examinations
2.1.1. Collection of samples:
One hundred samples of raw chicken breast and thigh (50 of each) "weighed about 250g/sample" were collected from an automatic poultry dressing plant in Ismailia city, Egypt. The collected samples were prepared as recommended by ISO 6887-1 (2017), then subjected to the following examinations:

2.1.2. Detection of E. coli was performed according to ISO 16649-2 (2001)
2.1.2.1. Serotyping of E. coli isolates was performed following Kok *et al.* (1996).
2.1.2.2. Detection of Salmonellae was performed according to ISO 6579-1 (2017).

2.2. *Genetic detection of E. coli fliC gene, and Salmonella fimA gene*
2.2.1. Genetic detection of E. coli fliC gene:
2.2.1.1. DNA extraction was performed according to Shah *et al.* (2009) using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) following manufacturer's recommendations. Briefly, 200 µl of the prepared sample suspension was incubated with ten µl of proteinase K and 200 µl of lysis buffer at 56^{O}C/10 min. After incubation, 200 µl of absolute ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

2.2.1.2. Oligonucleotide primer was purchased from Metabion (Germany) as listed in table (1).

2.2.1.3. PCR amplification was performed according to Fagan *et al*. (1999)
Primers were mixed in 25 µl reaction mixture consists of 12.5 µl PCR Master Mix (Takara, Japan), 1 µl of forward and reverse primers (20 pmol conc.), 4.5 µl of PCR grade water and 6 µl of DNA template. The reaction was performed in a calibrated adjusted 2720 thermal cycler.

2.3. Molecular detection of Salmonella fimA gene:
2.3.1. DNA extraction was performed according to Shah *et al.* **(**2009)
An overnight bacterial culture (200 µl) was mixed with 800µl of distilled water and boiled for 10 min. The resulting solution was mixed with 0.63ml 10m MNTPs (Ahmed, 2009).

2.3.2. Oligonucleotide primer was purchased from Metabion (Germany) as listed in table (2).

2.3.3. DNA amplification was performed according to Singh *et al.* (2013).
The bacterial genomic DNA extracts were amplified by PCR in a mixture of 25 µl. reaction mixture contained 13.25 sterile H_2O, 0.25 ml 10x buffer, 0.63 ml 10m MNTPs, 1 ml 25Mm $MgCl_2$, 1.25 µl primer F and primer R (20 pmol/ml conc.); and then, fill up to 25 µl PCR grade water.

Table 1 Primer sequences of *E. coli* O_{157}:H_7 *fliC* gene used for PCR identification system.

Target gene		Primers sequences (5'→3')	Amplified segment (bp)	Reference
E. coli O_{157}:H_7 *fliC*	F	GCGCTGTCGAGTTCTATCGAGC	625	Fratamico *et al.* (2000)
	R	CAACGGTGACTTTATCGCCATTCC		

Table 2 Primer sequences of Salmonella *fimA* gene used for PCR identification system

Target gene	Primers sequences (5'→3')	Amplified segment (bp)	Reference
Salmonella fim A	CCT TTC TCC ATC GTCCTGAA TGG TGT TAT CTG CCCGACCA	512	Cohen et al., (1996)

2.3.4. Analysis of the PCR Products
The genomic copies of PCR products were separated by action of electrophoresis on 1.5% agarose gel (AppliChem, Germany, GmbH) soaked in 1x TBE buffer at 25 °C passing 5V/cm electrical current. For gel analysis, twenty µl of the products was injected in each gel incision. 100 bp genetic ladders were utilized to verify the fragment size. The gel photo was captured by a gel documentation system and analyzed by computer software.

2.4. Statistical Analysis:
The recorded results were performed using the Analysis of Variance (ANOVA) test following Feldman *et al.* (2003).

3. RESULTS

3.1. Prevalence of E. coli
Results of the study verified detection of *E. coli* in six (6%) and nine (9%) breast and thigh samples, respectively. In addition, bacteriological and serotyping of the isolated *E. coli* strains revealed that 4 out of 15 isolated strains were *E. coli* O_{157}:H_7 as recorded in table (3).

Table 3 Prevalence of *E. coli* strains the isolated from the examined chicken samples of meat cuts

E. coli strains	Examined chicken meat cuts samples (n=50)					
	Chicken breasts isolates (n=6)		Chicken thighs isolates (n=9)		Total	
	No.	%*	No.	%*	No.	%**
Non O_{157}:H_7	4	8	7	14	11	11
O_{157}:H_7 (EHEC)	2	2	2	2	4	4

*percent calculated according to number of each examined samples (50).
**percent calculated according to total number of the examined samples (100)

Genetic confirmatory detection of *E. coli* O_{157}:H_7 revealed the presence of *fliC* gene band at 625bp as recorded in fig. (1). This showed that all the examined four isolates were positive for H_7 *fliC* gene with prevalence of 100%.

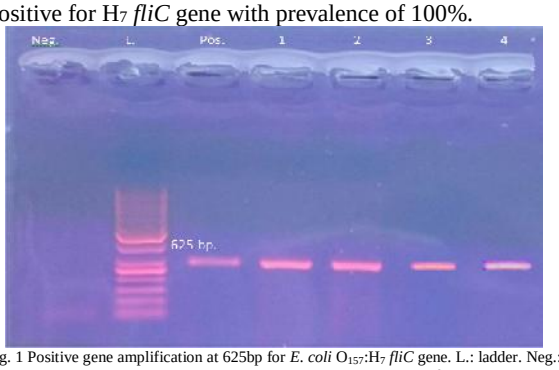

Fig. 1 Positive gene amplification at 625bp for *E. coli* O_{157}:H_7 *fliC* gene. L.: ladder. Neg.: Control negative. Pos.: Control positive. Lanes 1-4: positive isolates of O_{157}:H_7 at 625 bp.

3.2. Prevalence of Salmonellae

The presence of Salmonella was investigated using standard bacteriological techniques, then examined genetically by conventional PCR technique to detect presence of *fimA* gene in the isolates. Results of the present study revealed detection of Salmonella spp. in eleven (11%) of the examined samples represented by seven (14%) and four (8%) breast and thigh samples, respectively as recorded in table (4).

Table 4 Prevalence of Salmonellae isolated from the examined samples of chicken meat cuts

	Examined chicken meat cuts samples (n=50)					
	Chicken breast		Chicken thigh		Total	
	No.	%*	No.	%*	No.	%**
Salmonella species	7	14.0	4	8	11	11

*percent calculated according to number of each examined samples (50).
**percent calculated according to total number of the examined samples (100).

For confirmatory diagnosis of Salmonellae, *fimA* gene was investigated as recorded in Fig. (2), which showing that out of five examined isolates, four isolates carried *fimA* gene with prevalence of 80%.

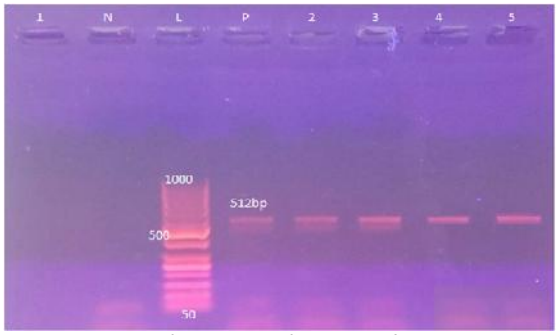

Fig. 2 Positive gene amplification at 512bp for Salmonella *fimA* gene. N: negative. P: positive. Lane 1: negative Salmonella strains for *fimA* gene. Lanes 2-5: Positive Salmonella strains for *fimA* gene

4. DISCUSSION

The PCR assays are considered as one the most specific, rapid, and diagnostic tools for microbial food poisoning detection, especially for ensuring the safety and quality of food.

Results achieved in table (3) and fig. (1) indicated that 100% of the identified *E. coli* O_{157}:H_7 serologically isolated from chicken meat samples were positive by application of PCR technique. Thus, there was complete agreement between the results of serological (traditional) methods and PCR technique for identification of *E. coli* O_{157}:H_7. These findings agree with those reported by Asensi and dos Reis *et al.* (2009), who reported that PCR matched with the results obtained by traditional classical methodology in less time and labor, made it a good alternative screening technique for screening and diagnosis of pathogenic foodborne contamination in a many samples such as imported and commercially produced poultry samples, getting benefit of rapid, specific and sensitivity.

In addition, the results of *E. coli* O_{157}:H_7 detection in raw chicken meat samples was previously reported by Saad *et al.* (2012), who found it with mean value of $1.4x10^2$ CFU/g in their examined samples; and Guran *et al.* (2017), who detected *E. coli* O_{157}:H_7 in 5(1.3%) chicken meat samples out of 375 examined samples. They also recorded that *fliC* gene in their examined samples.

Salmonella is a crucial reason behind foodborne diseases related to increased morbidity and mortality through the world (Scallan *et al.*, 2015). It was reported that foodborne Salmonella poisoning as the 2nd most recorded bacterial foodborne illness in the USA (Scallan *et al.*, 2011), where it causes about 22.2% of foodborne diseases in China as reported by Wang *et al.* (2007).

Results achieved in table (4) indicates the prevalence of Salmonellae in the examined breast and thigh samples. The obtained results can be compared with those recorded by Jorgensen *et al.* (2002) (25%) of the examined chicken meat cuts), Molla and Mesfln (2003) (21.1%) of the examined chicken meat), Tibaijuka *et al.* (2003) (12.3% in raw chicken meat), Atia (2018) (Salmonella was isolated from 8% and 20% of breast and thigh samples, respectively), and Elsisy (2019) (Salmonella was detected in 20 and 25% of the examined chicken breast and chicken thigh, respectively).

Results of molecular investigation of the presence of *fimA* gene in the examined Salmonella isolates were in agree with Akeem *et al.* (2017), and Zhengquan *et al.* (2020), who detected *fimA* gene in all of Salmonellae strains (24 and 151 isolates, respectively).

The specificity of the reaction is primarily dependent on careful selection of the oligonucleotide primers and primer annealing temperature.

5. CONCULSION

The results concluded that, the PCR is one of the most promising techniques available for rapid detection of foodborne and environmental microorganisms. Raw chicken meat cuts are exposed to many contamination ways through its production cycle. Detection of such bacteria (*E. coli* O_{157}:H_7 and Salmonella) indicated that raw chicken meats can possess a health hazards to the consumers, furthermore, it encourages following more hygienic practices during production and processing.

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